Susceptibility of the Stages of the Cattle Biting Louse (Mallophaga: Trichodectidae) to Juveth, an Insect Juvenile Hormone Analog

D.E. Hopkins and W.F. Chamberlain

Agr. Res. Serv., USDA, Kerrville, Texas 78028

ABSTRACT

When eggs of the cattle biting louse, *Bovicola bovis* (L.), were exposed to the vapors of or treated topically with juveth, an analog of the insect juvenile hormone, a supernumerary nymphs resulted. However, metamorphosis was affected only when the penultimate stage (3rd-instar nymph) was exposed to hormonal residues from the treated eggs. Latent or programmed effects did not occur.

We previously reported that *Bovicola limbata* (Gervais) treated topically in the first 2 days of the 3rd stage with juveth (ethyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate), a juvenile hormone analog, developed to atypical 4th-stage lice, but that those treated during the latter part of the 3rd stage developed to typical adults (Hopkins et al. 1970).

Latent effects that altered the adult metamorphosis of Hemiptera (Riddiford 1969, 1970) and Homoptera (White 1968) but left 1 or more immature stages apparently unchanged have reportedly occurred as a result of treatment of eggs or early instars with synthetic juvenile hormone or with analogs of synthetic juvenile hormone. A like result was observed in Lepidoptera (Riddiford and Williams 1967) after treatment of eggs.

Willis and Lawrence (1970) also observed metamorphic changes in late-stage *Oncopeltus fasciatus* after eggs were treated. They indicated that the effects were deferred, the result of the hormone's being progressively transferred through the integument as each molt occurred, but not the result of latent action.

We report here that treating eggs of *B. bovis* (L.), the cattle biting louse, with juveth resulted in atypical phenotypes (Hopkins et al. 1970) only when 3rd- or penultimate-stage nymphs were reared on diet that contained residues from treated eggs and mohair.

In preliminary tests, we observed that atypical 4th-instar phenotypes developed from eggs of *B. bovis* exposed to juveth vapor. However, we also observed that atypical phenotypes developed on diet coated with 50 ppm of juveth only if the lice were reared on the diet during the 3rd nymphal stage. Those that were reared on the diet during only the 1st or only the 1st and 2nd stages developed to typical adults.

This inconsistency of results when eggs and nymphal forms were treated and the possibility that latent effects might occur was investigated by a variety of tests. The test eggs were taken from our colony of *B. bovis* which is fed a diet of the surface scrapings of cowskin (Hopkins and Chamberlain 1972). The tests were always conducted at 72% RH and 37±1.5°C except that during the period of exposure to vapor the eggs were in vials that had been sealed at room RH.

For the exposure to juveth vapor, the juveth was dissolved in glass-distilled acetone (1 mg/200 μl), and 200 μl of the solution was pipetted into a 20-ml glass vial. The lower two-thirds (ca.) of the inner surface of the vial was coated with the juveth by tilting and rotating the vial by hand until the acetone had evaporated. Then bundles of mohair (10-15 pieces each about 1.5 cm long) each with 20 attached 0- to 1-day-old louse eggs were secured to the center of the cork-backed metal foil liner of the screw cap of

the vial with two 2X8-mm strips of masking tape, and the cap was screwed onto the vial (care was taken that the eggs or hair did not touch the treated surface). A similar untreated vial contained bundles with control eggs. On the 6th day of exposure (the eggs began to hatch the 7th day), the eggs and hair were removed from the vials, and each bundle was dipped once in and flushed twice with acetone in an attempt to remove any juveth that might have been deposited on the surfaces of the eggs or the hair. The controls were treated similarly. The hair and eggs were then placed at 3 conditions to determine which stage(s) were susceptible to the effects of the juveth: (1) a bundle with treated eggs was placed in a 0.5-dr glass shell vial with 20 mg of diet; (2) a bundle with control eggs was placed in a similar vial along with another bundle containing 20 vapor-treated eggs that had been killed by freezing (held at -5°C for 1-2 hr); (3) a bundle with control eggs was placed in a vial with diet. Each of these vial tests was replicated 4 times. The vials were then held in normal rearing conditions. At 6 days posthatch, the unhatched eggs, egg shells, and mohair were removed from all vials, and the nymphs were left to feed on the diet for 2 more days.

At 8 days posthatch, most nymphs were 2nd instars, and the numbers alive were recorded. Some late 2nd instars that had hatched from treated eggs and some that had hatched from untreated eggs subsequently confined with killed vapor-treated eggs were placed on new diet in new vials. The remainder in the test vials and those in the control vials were allowed to finish development on original diet. When all lice had molted to the 4th instar or had died, the numbers of typical and atypical phenotypes in each vial were recorded.

As shown in Table 1, atypical (nymphoid) 4th instars developed only from 3rd instars that had been left on diet exposed to treated eggs and hair, and latent effects were not demonstrated.

In a 2nd test, eggs of *B. bovis* were treated topically with juveth in acetone solution in a manner similar to the way Riddiford (1970) treated the eggs of *Pyrrhocoris apterus* and *Oncopeltus fasciatus*. Three acetone solutions of juveth were prepared, and each was applied to separate groups of twenty 0- to 1-day-old and 6- to 7-day-old eggs (2 replications of each age and each solution) with micrometer-actuated syringes: 0.52 μl of 0.5%/egg with a 0.25-ml syringe and 0.05 μl of 1 or 2%/egg with a 100-μl syringe. Also, 2 control groups of each age (20/group) were treated with each amount of acetone alone. Each group was placed in a

Table 1. Phenotypes of 4th-Instar *B. bovis* Resulting from Eggs Treated with Juveth and Subsequent Rearing on Diet Containing Hormonal Residues and Control Diets.

Treatment	Diet of Nymphs Beginning 8 Days Posthatch	No. and Phenotype of 4th Instars
	Vapor treatment	
Treated	15 on original diet	12 nymphs
	12 on fresh diet	11 adults
Untreated[a]	30 on original diet	27 nymphs
	27 on fresh diet	26 adults
Untreated	70 on original diet	67 adults
	Topical treatment	
0.5 μg/egg	16 on original diet	12 nymphs
	14 on fresh diet	11 adults
Untreated	20 on original diet	18 adults
	14 on old[b] diet	8 nymphs

[a]Held with treated frozen eggs and mohair for last day of egg stage and first 6 days of nymphal stage.

[b]Diet that had been in a vial with treated eggs for about 9 days.

0.5-dr glass shell vial with diet and allowed to hatch and develop for 8 days; then new diet in a new vial was provided for some of the lice while the remainder were left on the original diet, and records were taken when all lice had molted to the 4th instar or died. In addition, some diet that had originally been exposed to treated eggs and mohair was used as food for some 2nd-instar nymphs from the acetone-treated controls.

In each test, 0- to 1-day-old eggs treated topically with juveth failed to hatch, but all controls hatched. The hatchability of treated 6- to 7-day-old eggs equaled that of the controls, but the rate of survival of nymphs was low (<50%) for the higher doses. The results of the test with 0.05 μl of 1%/egg or 0.5 μg/egg are presented in Table 1 (the results of the other tests were similar, but survival was best in this test). Obviously latent effects from treated eggs were not demonstrated, but atypical phenotypes developed when lice were exposed to contaminated diet.

For 50 ppm of juveth, which was an effective dose, to be present in the 20 mg of diet used in the vapor tests, at least 1000 ng would have to be transferred somehow from the wall of the vial to the eggs and hair and subsequently, despite the washing of the eggs and hair, to the diet; we placed 1 mg (or 1000X that amount) of juveth on the wall of the vial. In the tests of topical treatment, we used 10X (10,000 ng/20 eggs) that amount per vial. We feel that these amounts of juveth were adequate so at least 50 ppm could have been transferred to the diet.

We conclude that, in this species of Mallophaga, treatment with an active juvenile hormone analog produces a response only if the lice are treated during the 3rd instar. The occurrence of latent or deferred effects when hemipteran and lepidopteran eggs were treated with an analog of JH and not when mallophagan eggs were treated may be the result of fundamental differences in the respective metamorphoses. However, hormonally active materials usually act at such low concentrations that the carryover of minute amounts affects results.

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